



DNA Purification Products

genPURE PCR™

PCR Purification System

User guide

Introduction

genPURE PCR Purification Systems, available in column, 96 well and 384 well plate format, make up part of Genetix's genPURE range of DNA purification products. They have been specially developed for the fast, effective purification of PCR products using different formats accustomed for different needs. They allow single or multiple parallel purification of PCR product samples in 10-15 minutes, using either a vacuum or centrifuge purification system. The columns also enable the extraction and purification of DNA fragments from Agarose gels.

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For Research Use Only.

Storage Instructions

Store all components at room temperature and shake buffers before use.

Kit Components

genPURE PCR 96	(2)	(10)
Binding Buffer	110ml	2 _ 250ml
Wash Buffer (Add 80ml or 200ml 100% ethanol to each bottle)	2 _ 20ml	3 _ 50ml
Elution Buffer	30ml	2 _ 50ml
genPURE PCR 96 well Filter Plate	2	10
Deep 96 Well Wash Plate	2	10
96 well Collection Plate	2	10
Adhesive plate seal	2	10
genPURE PCR 384	(2)	(10)
Binding Buffer	120ml	3 _ 200ml
Wash Buffer (Add 80ml or 200ml 100% ethanol to each bottle)	50ml	3 _ 50ml
Elution Buffer	30ml	125ml
genPURE PCR 384 well Filter Plate	2	10
384 well Wash Plate	2	10
384 well Collection Plate	2	10
Adhesive plate seal	2	10
genPURE PCR Clean up and Gel Extraction Columns	(50)	(250)
Binding Buffer	35ml	2 _ 90ml
Wash Buffer (Add 60ml or 150ml 100% Ethanol to each bottle)	15ml	2 _ 37.5ml
Elution Buffer	10ml	50ml
genPURE Columns	50	250
Collection Tubes	50	250

Materials Supplied by the User

Vacuum Manifold, Centrifuge or Microcentrifuge
100% Ethanol

Technical Note

Several different vacuum manifolds are available for use with the genPURE 96 & 384 PCR kits. We suggest using one of the following:

Genetix Vacuum Manifold (Available soon)

Whatman UniVac (Cat # 7705-0102).

Eppendorf Perfect Vac Manifolds, single or quad (order # 1-959100 / 1-959102)

QIAGEN's QIAVac 96, however, is not compatible.

Preparations

Prior to using the genPURE PCR kits ensure that you add the amount of ethanol indicated on each Wash Buffer bottle and mark that it has been done by checking the box on the label.

Relationship between inches Hg and other pressure measurements and the Centrifugal Force used in all steps of the alternative centrifuge protocol

15 Inches Hg	50.8	Kpa
	380	Torr
	0.50	atm
	7.37	Psi
	38.1	cm Hg
	508	mbar

Centrifugal Force	1250g	Approx 3000rpm
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$$\text{rpm} = \sqrt{750/1.12r} \text{ (r = radius of rotor in mm)}$$

genPURE PCR-96

Vacuum Method

- 1.) To each PCR reaction (10µl-100µl), add five times the amount of binding buffer and mix well by pipetting up and down (e.g to a 50µl PCR reaction, add 250µl Binding Buffer). For the larger volume PCR reactions you may need to transfer the PCR product to a separate deep well plate first and carry out the mixing there.
- 2.) Set-up the vacuum manifold as directed by supplier. Place the deep well wash plate provided into the vacuum manifold and place the genPURE filter plate on top. Connect the vacuum source.
- 3.) If a separate mixing plate was used in step 1, now transfer the sample and Binding Buffer mixes to the wells of the genPURE filter plate. Apply the vacuum at a pressure of –15 inches Hg for approximately 2 minutes, or until all liquid has been visibly removed.

This binds the PCR products to the membrane of the filter plate.

- 4.) Discard the buffer from the wash plate and return it to the vacuum manifold.
- 5.) To each well used, add 750µl of the Wash Buffer and apply a vacuum of –15 inches Hg. (NB Make sure you have added the correct amount of ethanol to the wash buffer bottle.) Switch off the vacuum source only when all of the Wash Buffer has passed through the plate. Again this usually takes about 2 minutes but longer vacuum times may be necessary.

This washes the PCR product of its primers, unincorporated nucleotides etc.

- 6.) Discard the buffer once again and return the plates to the manifold.
- 7.) Apply maximum vacuum (at least –20 inches Hg) for an extra 5 minutes to remove any residual ethanol left from the previous step. For less powerful vacuums, the duration of this step may need to be increased accordingly to ensure removal of all the ethanol. After the 5 minutes, take the filter plate off the manifold and rap firmly on some clean tissue until no liquid is visible on the tissue.

This step is necessary as ethanol can interfere with any further reactions down the line.

- 8.) Remove the wash plate and replace it with the shallow well, low volume, collection plate provided.

Depending on the type of vacuum manifold used, it may be necessary to decrease the gap between the genPURE filter plate and the collection plate using additional microtitre plates. This is to prevent cross contamination between wells due to potential splashing of the eluate.

- 9.) To elute the DNA, add 30µl of Elution Buffer to each well and leave the stand for at least 1 minute. Apply the vacuum at –15 inches Hg for 1 minute.

The purified PCR product can also be eluted with dH₂O or TE. To increase your final yield, a second 30µl elution can be performed. This will however, decrease the final concentration.

Centrifuge Method

- 1.) To each of your PCR reactions (10µl-100µl), add five times the amount of Binding Buffer and mix well by pipetting up and down (e.g to 50µl of PCR reaction volume, add 250µl Binding Buffer). For the larger volume PCR reactions you may need to transfer the PCR product to a separate deep well plate first and carry out the mixing there.
- 2.) Once the reactions have been added to the genPURE filter plate place it on top of a deep well wash plate provided.
- 3.) Centrifuge at 1250g for 2 minutes.

This binds the PCR products to the membrane of the filter plate.

- 4.) Discard the buffer from the wash plate and replace it back under the genPURE filter plate.
- 5.) To each well used, add 750µl of the Wash Buffer and centrifuge for 2 minutes at 1250g. (NB Make sure you have added the correct amount of ethanol to the wash buffer bottle.)

This washes the PCR product of its primers, unincorporated nucleotides etc.

- 6.) Discard the buffer once again from the wash plate and replace it back under the genPURE filter plate.
- 7.) Centrifuge at maximum speed (no greater than 2250g, approx. 4000rpm) for an extra 5 minutes to remove any residual ethanol left from the previous step. After the 5 minutes, take the filter plate off the manifold and rap firmly on some clean tissue until no liquid is visible on the tissue.

This step is necessary as ethanol can interfere with any further reactions down the line.

- 8.) Remove the wash plate and replace it with the shallow well, low volume, collection plate provided.
- 9.) To elute the DNA, add 30µl of Elution Buffer to each well and leave the stand for at least 1 minute. Centrifuge at 1250g for 2 minutes.

The purified PCR product can also be eluted with dH₂O or TE. To increase your final yield, a second 30µl elution can be performed. This will however, decrease the final concentration.

genPURE PCR-384

Vacuum Method

- 1.) To each PCR reaction (5-15µl), add five times the volume of Binding buffer and mix well by pipetting up and down (e.g. to a 15µl reaction add 75µl of Binding buffer). Alternatively you may add the appropriate Binding buffer to each well of the filter plate and then add your PCR reactions to it. Be sure to mix well by pipetting up and down several times.
- 2.) Set up the vacuum manifold as directed by supplier. Place the wash plate provided into the vacuum manifold and place the genPURE filter plate on top. Connect to the vacuum source.
- 3.) Load the sample and binding mixes into wells of the genPURE filter plate and apply the vacuum for approximately 2 minutes at –15 inches Hg or until all liquid has been visibly removed.

This will bind the PCR samples to the membrane of the filter plate.

- 4.) Discard the buffer from the wash plate. Return the wash plate to the vacuum manifold.
- 5.) To each well used, add 90µl of the wash buffer (making sure that you have added the correct amount of ethanol to the wash buffer) and apply a vacuum of –15 inches Hg. Switch off vacuum source when all buffer has passed through the plate or after approximately 2 minutes. Discard the buffer. A second 90µl wash can be carried out at this stage.

This washes the PCR product of its primers, unincorporated nucleotides etc.

- 6.) Once the buffer has been discarded, return the plates to the manifold.
- 7.) Apply maximum vacuum (at least –20 inches Hg) for an extra 5 minutes to remove any residual ethanol left from the previous step. For less powerful vacuums, the duration of this step may need to be increased accordingly to ensure removal of all the ethanol. After the 5 minutes, take the filter plate off the manifold and rap firmly on some clean tissue until no liquid is visible on the tissue.

This step is necessary as ethanol can interfere with any further reactions down the line.

- 8.) Remove the wash plate and replace it with the collection plate.
- 9.) To elute the DNA, add 15µl elution buffer to each well and leave the plate to stand for at least 1 minute. Apply the vacuum for 1 minute at –15 inches Hg.

The purified PCR product can also be eluted with dH₂O or TE. To increase the final yield, a second 15µl elution can be performed, but will result in a more diluted final sample.

Centrifuge Method

- 1.) To each of your PCR reactions (5µl-15µl), add five times the amount of Binding Buffer and mix well by pipetting up and down (e.g to 15µl of PCR reaction volume, add 75µl Binding Buffer). Alternatively you may add the appropriate Binding buffer to each well of the filter plate and then add your PCR reactions to it. Be sure to mix well by pipetting up and down several times.
- 2.) Once the reactions have been added to the genPURE filter plate place it on top of a wash plate provided.
- 3.) Centrifuge at 1250g for 2 minutes.

This binds the PCR products to the membrane of the filter plate.

- 4.) Discard the buffer from the wash plate and replace it back under the genPURE filter plate.
- 5.) To each well used, add 90µl of the Wash Buffer and centrifuge for 2 minutes at 1250g. (NB Make sure you have added the correct amount of ethanol to the wash buffer bottle.) Discard the buffer. A second 90µl wash can be carried out at this stage.

This washes the PCR product of its primers, unincorporated nucleotides etc.

- 6.) Once the buffer has been discarded from the wash plate and replace it back under the genPURE filter plate. Return the plates to the centrifuge.
- 7.) Centrifuge at maximum speed (no greater than 2250g, approx. 4000rpm) for an extra 5 minutes to remove any residual ethanol left from the previous step. After the 5 minutes, take the filter plate off the manifold and rap firmly on some clean tissue until no liquid is visible on the tissue.

This step is necessary as ethanol can interfere with any further reactions down the line.

- 8.) Remove the wash plate and replace it with a collection plate provided.
- 9.) To elute the DNA, add 15µl of Elution Buffer to each well and leave the stand for at least 1 minute. Centrifuge at 1250g for 2 minutes.

The purified PCR product can also be eluted with dH₂O or TE. To increase your final yield, a second 15µl elution can be performed. This will however, decrease the final concentration.

genPURE PCR Clean-up & Gel Extraction Columns

PCR Clean-up Protocol

- 1.) For PCR and DNA solutions of $\leq 100\mu\text{l}$, add $500\mu\text{l}$ of Binding buffer and vortex the tube briefly to mix.

For any sample of PCR or DNA solution exceeding $100\mu\text{l}$ in volume, add 5 times the amount of binding buffer. (e.g. $200\mu\text{l}$ sample add $1000\mu\text{l}$ Binding buffer).

The maximum volume of the genPURE column is $750\mu\text{l}$, if the sample/binding buffer mix volume exceeds this load the $750\mu\text{l}$, proceed to step 2 then add the remaining mix and repeat the spin.

- 2.) Insert one of the genPURE spin columns into a collection tube and transfer your sample, containing the Binding buffer, to the column. Then spin at maximum speed ($12000 \sim 14000g$) for 1 minute.
- 3.) Remove the genPURE column from the collection tube, discard the buffer from the collection tube and replace the genPURE column. Then add $700\mu\text{l}$ of wash buffer to the column and spin for approximately 2 minutes at maximum speed, ($12000 \sim 14000g$).

For fluorescent sequencing, an additional wash is recommended.

- 4.) Remove the column and discard the buffer from the collection tube. Replace the column into the collection tube and re-spin for 3 minutes at the maximum speed to remove any residual trace of ethanol.

For fluorescent sequencing a recommended step to make quite sure the ethanol has gone is to place the sample in a $37-60^{\circ}\text{C}$ oven for up to 10 minutes.

Do not leave the column in the oven for any longer.

- 5.) Remove the genPURE column and place in a new collection tube. To elute the DNA, add $30\mu\text{l}$ of Elution buffer to the centre of the membrane and leave stand for 1 minute. Then spin at the maximum speed for 1 minute. Store the eluted DNA at -20°C . dH_2O or TE buffer may also be used to elute the DNA

When eluting with dH_2O make sure the pH is within 7.0 - 8.5, lower pH may cause lower DNA recovery.

For fluorescent sequencing use dH_2O only to elute.

If checking DNA on Agarose gel after elution in dH_2O , add 1 volume of elution buffer in DNA/loading dye mixture, or DNA may run faster than expected.

Gel Extraction Protocol

- 1.) Firstly excise the desired band of DNA from the Agarose gel trying to take as little Agarose as possible. Then weigh it, slice it into smaller sections and place it into a microcentrifuge tube.
- 2.) Add an equal volume of binding buffer to the excised gel relative to its weight (e.g. 100µl of binding buffer to 100mg of gel). Melt at 60°C until the gel has completely dissolved.

For >2% gel, add 2-3 volumes of binding buffer.

More binding may be added to speed up the melting process – DNA recovery will not be affected either way.

- 3.) Place a genPURE column into a collection tube and transfer the melted solution into the column and spin at maximum speed for 1 minute. Once spun, remove the column from the collection tube, discard the eluted buffer and replace the column.
- 4.) Add 500µl of binding buffer to the column and spin at maximum speed for 1 minute. This step will remove any residual Agarose, which may inhibit enzymatic reactions further down the line. Once spun, discard the buffer.
- 5.) Add 700µl of wash buffer to the column and spin at maximum speed for 1 minute.

An additional wash is recommended for fluorescent sequencing.

- 6.) Remove the column and discard the buffer from the collection tube. Replace the column into the collection tube and re-spin for 3 minutes at the maximum speed to remove any residual trace of ethanol.

For fluorescent sequencing a recommended step to make quite sure the ethanol has gone is to place the sample in a 37- 60°C oven for up to 10 minutes.

Do not leave the column in the oven for any longer.

- 7.) Remove the genPURE column and place in a new collection tube. To elute the DNA, add 30µl of Elution buffer to the centre of the membrane and leave stand for 1 minute. Then spin at the maximum speed for 1 minute. Store the eluted DNA at –20°C. dH₂O or TE buffer may also be used to elute the DNA

When eluting with dH₂O make sure the pH is within 7.0 - 8.5, lower pH may cause lower DNA recovery.

For fluorescent sequencing use dH₂O only to elute.

If checking DNA on Agarose gel after elution in dH₂O, add 1 volume of elution buffer in DNA/loading dye mixture, or DNA may run faster than expected.